

WHAT IS CLAIMED IS:

1. (currently amended) A biofuel cell for generating electricity using a fuel fluid comprising:

a substrate;

5 a cathode supported by the substrate and capable of a reaction to reduce an oxidant in the presence of electrons to form water;

an anode supported by the substrate and capable of a reaction to oxidize the fuel fluid;

at least one of the anode and cathode including an enzyme for use in carrying out its respective reaction; **and either**

10 **(i) at least one of the anode and cathode being formed for flow of the fuel fluid therewithin for use in producing an electrical current;**

**(ii) the cathode comprising an enzyme immobilization material comprising a micellar or inverted micellar structure; or**

15 **(iii) at least one of the anode and cathode comprising a width less than about 200  $\mu\text{m}$  and at least one surface having an irregular, three dimensional topography capable of inducing convective flow of the fuel fluid and/or oxidant over said surface.**

2. (currently amended) The biofuel cell of claim 1, wherein the anode comprises

(a) an electron conductor;

5 (b) an electron mediator, the reduced form of the electron mediator being capable of releasing electrons to the electron conductor;

(c) at least one enzyme capable of reacting with the oxidized form of the electron mediator and the fuel fluid to produce an oxidized form of the fuel fluid and a reduced form of the electron mediator;

(d) an enzyme immobilization material capable of immobilizing and stabilizing  
10 the enzyme, the material being permeable to the fuel fluid and the electron  
mediator, **and optionally, the material comprises the electron mediator.**

3. (cancelled).

4. (currently amended) The biofuel cell of claim 1, wherein the anode  
comprises

(a) an electron conductor;

(b) an electron mediator;

5 (c) at least one enzyme capable of reacting with the oxidized form of the  
electron mediator and the fuel fluid to produce an oxidized form of the fuel fluid and  
a reduced form of the electron mediator;

(c) an enzyme immobilization material **optionally comprising the electron  
mediator and/or an electrocatalyst, the material being** capable of immobilizing  
10 and stabilizing the enzyme, the material being permeable to the fuel fluid **and the  
electron mediator**; and

(d) an electrocatalyst adjacent the electron conductor, an oxidized form of the  
electrocatalyst being capable of reacting with the reduced form of the electron  
mediator to produce an oxidized form of the electron mediator and a reduced form  
15 of the electrocatalyst, the reduced form of the electrocatalyst being capable of  
releasing electrons to the electron conductor.

5. - 6. (cancelled).

7. (currently amended) The biofuel cell of **any one of claims 2-3 claim 2**  
wherein the anode's electron mediator comprises pyrroloquinoline quinone (PQQ),  
phenazine methosulfate, dichlorophenol, indophenol, short chain ubiquinones, or  
potassium ferricyanide.

8. - 10. (cancelled).

11. (currently amended) The biofuel cell of claim ~~10~~ **2** wherein the ~~oxidoreductase~~ **enzyme** comprises an oxidoreductase that acts on the CH-OH group or CH-NH group, a dehydrogenase, alcohol dehydrogenase, aldehyde dehydrogenase, formate dehydrogenase, formaldehyde dehydrogenase, glucose  
5 dehydrogenase, glucose oxidase, lactic dehydrogenase, lactose dehydrogenase, pyruvate dehydrogenase, or a PQQ-dependent dehydrogenase.

12. (cancelled).

13. (currently amended) The biofuel cell of ~~any one of claims 1-11~~ **claim 1**, wherein the cathode comprises

- (a) an electron conductor;
- (b) at least one enzyme capable of reacting with a reduced form of an  
5 electron mediator and an oxidant to produce an oxidized form of the electron mediator and water; and
- (c) an enzyme immobilization material comprising **either the electron mediator**, an electrocatalyst, **or the electron mediator and an electrocatalyst**,  
10 the enzyme immobilization material being capable of immobilizing and stabilizing the enzyme, the material being permeable to the oxidant, an oxidized form of the electrocatalyst being capable of gaining electrons from the electron conductor to produce a reduced form of the electrocatalyst ~~which~~ **that** is capable of reacting with an oxidized form of the electron mediator to produce a reduced form of the electron mediator and an oxidized form of the electrocatalyst.

14. (currently amended) The biofuel cell of ~~any one of claims 1-11~~ **claim 1**, wherein the cathode comprises

- (a) an electron conductor;

(b) at least one enzyme capable of reacting with a reduced form of an  
5 electron mediator and an oxidant to produce an oxidized form of the electron  
mediator and water; and

(c) an enzyme immobilization material **optionally comprising the electron**  
**mediator**, the enzyme immobilization material being capable of immobilizing and  
stabilizing the enzyme, the material being permeable to the oxidant, an oxidized  
10 form of the electron mediator being capable of gaining electrons from the electron  
conductor to produce a reduced form of the electron mediator.

15. - 16. (cancelled).

17. (currently amended) The biofuel cell of claim ~~46~~ **12** wherein the  
~~oxidoreductase~~ **enzyme** comprises a laccase, an oxidase, a glucose oxidase, an  
alcohol-based oxidase, a cholesterol-based oxidase, an oxygen oxidoreductase, or  
a bilirubin oxidase.

18. - 33. (cancelled).

34. (currently amended) The biofuel cell of ~~any one of claims 1-32~~ **claim 1**  
wherein the fuel fluid and ~~or~~ the oxidant is moved through the biofuel cell at a flow  
rate of between about 0.01  $\mu\text{L}/\text{min}$  and about 10  $\mu\text{L}/\text{min}$ .

35. - 37. (cancelled).

38. (currently amended) The biofuel cell of ~~any one of claims 2-37~~ **claim 2**  
wherein the enzyme immobilization material comprises a micellar or inverted  
micellar structure.

39. - 44. (cancelled).

45. (currently amended). An electrode ~~for use in the biofuel cell of any of claims 1-44, the electrode~~ comprising an electron conductor having a width less than about 200  $\mu\text{m}$  and at least one surface having an irregular, three dimensional topography capable of inducing convective flow of the fuel fluid over said surface.

46. - 47. (cancelled).

48. (original) The electrode of claim 45, wherein the electrode has a width of between about 10  $\mu\text{m}$  and 50  $\mu\text{m}$ .

49. - 50. (cancelled).

51. (original) The electrode of claim 45, wherein the electrode comprises a carbon-based ink.

52. (cancelled).

53. (original) An electrode comprising an electron conductor having an effective surface area that is at least 1.5 times greater than its geometric surface area, wherein one dimension of the electrode is less than 100  $\mu\text{m}$ .

54. - 60. (cancelled).

61. (original) A method for forming an electrode for use in a biofuel cell, the method comprising

forming at least one electrical connector on a substrate;

forming at least one microchannel in a non-conductive casting mold

5 comprised of a material that will not passivate the electrode and can be reversibly sealed to the substrate;

adhering the casting mold to the substrate;

flowing an electron conductor solution through the microchannels; and  
curing the electron conductor solution to form the electrode.

62. (original) The method of claim 61, wherein the microchannels in the casting mold are formed using soft lithography.

63. (cancelled).

64. (original) The method of claim 61, wherein the substrate is flat.

65. (cancelled).

66. (original) The method of claim 61, wherein the method further comprises removing the casting mold and replacing it with a gas-permeable mold comprising larger microchannels.

67. (cancelled).

68. (original) The method of claim 66, wherein the microchannels in the gas-permeable mold are formed using soft lithography.

69. (cancelled).

70. (original) The method of claim 61, wherein the electron conductor solution comprises a carbon-based ink.

71. (cancelled).